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ORIGINAL ARTICLE

Effect of Calcium Phosphate Granules on the Repair of Femoral Bone Defects in Rabbits

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ABSTRACT
Segmental bone loss due to trauma, infection, and tumor resection and even non-union results in the vast demand for replacement and restoration of the function of the lost bone. The objective of present study was to evaluate effect of Calcium Phosphate granules on the healing of femoral bone defects in rabbits. Ten New Zealand white rabbits were used, of male gender, aged around 24 week and weighing 3.3-3.5 kg. Then animals were divided into two groups (control and experiment), with five rabbits in each. At the end of the experiment histopathologic and Histomorphometric parameters were evaluated. Histopathologic and Histomorphometric evaluations in present study showed that calcium phosphate granules improve osteogenesis in the defect area but more other researches are need yet.
Key words: Calcium Phosphate granules, femoral bone defects, rabbits.

INTRODUCTION

Segmental bone loss due to trauma, infection, and tumor resection and even non-union results in the vast demand for replacement and restoration of the function of the lost bone [1]. The treatment of these defects is the most challenging problem; many researchers are trying to find materials that can improve bone healing [2]. Autogenous bone, typically from the iliac crest, remains the preferred source of bone for grafting. However, disadvantages of autologous bone grafting that include limited supply, chronic pain, nerve damage, and wound complications [3, 4].
For preventing of these limitations, nowadays, such other materials include bone grafts are used as artificially or allogeneically. These materials have either osteoinductivity or osteoconductivity activity as an agent for transferring the conductor proteins of bone [2,5,6].
Many types of bone filling materials such as different types of calcium phosphate have been developed and have played critical roles in bone repair [5]. Calcium phosphate used as an alternative bone substitute in bone grafting [7]. It has been reported that calcium phosphate has excellent osteoconduction and resorbability when filling the bone defect [8, 9, 10, 11]. With regard to shape, both block and granular of calcium phosphate have been available. In this study, we evaluated histopathology and histomorphometry effect of calcium phosphate granules on the healing of bone defect in rabbit models.

MATERIALS AND METHODS

Animals
Ten New Zealand white rabbits were used, of male gender, aged around 24 week and weighing 3.3-3.5 kg. The animals were obtained from the Central Animal Laboratory of Islamic Azad University, Tabriz Branch. All animals were kept in individual cages during the whole experimental period, under similar conditions at 18-24^{o}C in a 12-hr dark-light cycle and maintained with unlimited amounts of standard laboratory pellet diet and water ad libitum. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care, and our ethical committee on animal care approved the protocol. Animals were divided into two groups (control and experiment), with five rabbits in each.
Calcium Phosphate granules were made of Kasios TCH Co. and were prepared as biophasic and had basis of tricalciumphosphate and hydroxyapatite. The component used for producing these products as given below:

75% hydroxylapatite ± 5% $Ca_{10}(PO_4)_6(OH)_2$
25% tricalcium phosphate ± 5%$Ca_3(PO_4)_2$

Granules used had 2-3 mm length and was provided as cubic. These granules have a porosity of 70% and the size of each pore was 200 to 500 micrometers and had resistance about 1 to 5 MPa according to the manufacturer's brochure.

Surgical procedure

All animals were submitted to surgery in both femurs; under general anesthetics by intramuscular injection of Xylazine/Ketamine (Ketamine 10%, Alfasan, Woerden-Holland, 50mg/kg and Xylazin 2%, Alfasan, Worden-Holland, 5mg/kg) and femurs was routinely asepsis. An incision was performed on femoral bone site. The muscle was dissected bluntly to complete exposure of the bone surface. Two monocortical perforations of 3mm in diameter were made with low-speed dental bit, saline-cooled in a stepwise fashion in left femurs of each animal next to its diaphysis. In control group defects were left empty. In experimental group each perforation was filled with calcium phosphate granules. The muscle attachment was sutured with simple stitches using Vicryl 4.0 suture with nontraumatic needle, and skin was sutured with silk suture 4.0, being removed at seven days after surgery. The animals received antibiotics Enrofloxacin 2.5% intramuscular in the dose of 2.5ml/Kg of body weight for five days after surgery, and anti-inflammatory Banamine (Flonexin Meglumine) injected in the dose of 1.1 mg/Kg of body weight for three days after surgery by applications in the muscle.

Histopathology and histomorphometry evaluation

Rabbits were euthanized with an intravenous injection of an over dosage of Thiopental sodium, causing a quick and painless death, at 45days postoperative. The bone piece with the perforation was removed and fixed in 10% neutral buffered formalin during five days, for fixation; then dehydrated in 10% EDTA. Finally, they were embedded in paraffin. Serial sections were cut and stained with Haematoxylin and Eosin (H&E) method and used for light microscopic examination under a Nikon microscope (ECLIPSE E200, Japan) to histopathology and histomorphometry evaluation. Histomorphometry analysis were performed by linear measurements through Intersection Latticed Lines using an ocular Latticed Lens comprising 100 cross points to determine the percentage of the defect that was occupied by 1) bone marrow, 2) woven bone and 3) lamellar bone. Thus, at a magnification of 40×, the various components were identified using a mouse cursor (12). Bone marrow was identified as a tissue that included adipocites, while connective tissue was defined by the presence of fibroblasts and collagen fibers. Morphometrical analysis was performed and the normal percentage of lamellar bone, woven bone and bone marrow was assessed.

Statistical analysis

Statistical evaluation of data was performed using the software package SPSS 18 (SPSS Inc., Chicago, IL). Data are expressed as the mean±SEM for each group. Statistical differences between groups were evaluated with T-test to analyze histomorphometric data among groups. The significant level was set at $p<0.05$.

RESULTS

Histopathology Results:

Histopathology findings of induced defect in the middle part of femoral diaphysis in control group showed that defect has been blocked by thin layer of bone. Primitive bone which has been made newly has filled bone marrow. Our evaluations in this group show that immature bone with wide spaces of bone marrow has consist in the defect area and newly made immature bone tissue has covered by active osteoblasts that indicate an active osteogenesis. These osteoblasts made first lamellar bone on old made spicules. At this time, trabeculae have marrow of immature bone which has covered by lamellar bone. Lack of production of organic bone matrix and its calcification prevents of compact bone (figs 1 and 2).

Assessment of results obtained from experimental group shows that induced defect in the middle part of femoral diaphysis has been filled by newly formed bone. Observing of lot of lamellar bone in the healing area indicates a regenerative process. The amounts of emerged bone in this group significantly are more than control group. Active osteogenesis with calcification in this group yields

to compaction in the new bone mass. Increase in remodeling of newly formed bone results in formation of hovers system. Sectional evaluations show that, 45 days after surgery, quantities of calcium phosphate granules seen yet (figs 3 and 4).

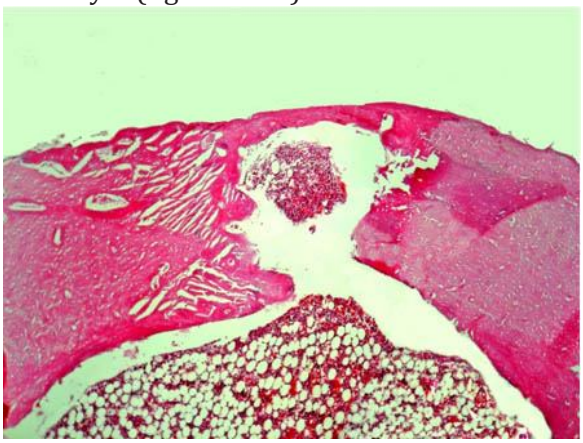

Fig 1: microscopic view from healing area in the middle part of femoral diaphysis from control group. A thin layer of primitive compact bone has occupied outer layer of bone defect and has filled bone marrow. Impaired organic bone matrix, osteoid and its deposition and calcification prevent from compaction of bone mass. H&E, 40x.

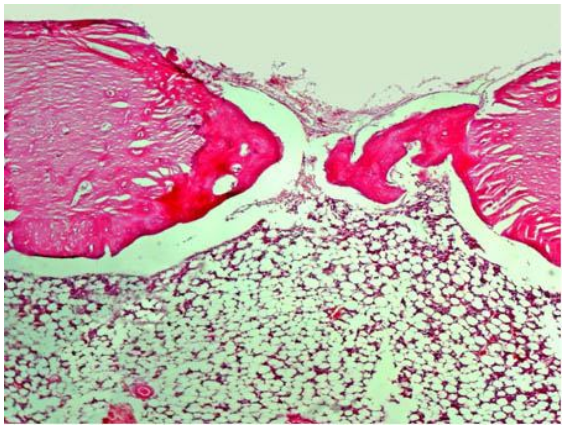

Fig 2: microscopic view from healing area in the middle part of femoral diaphysis from control group. Newly formed woven bone with wide spaces between them has occupied significantly defect area. H&E, 40x.

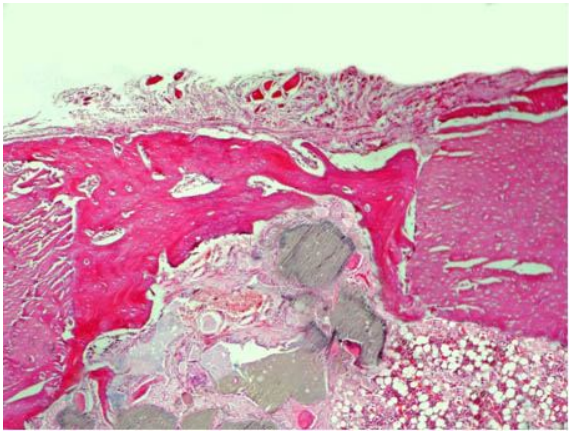

Fig 3: microscopic view from healing area in the middle part of femoral diaphysis from group treated with Calcium Phosphate Granules. Defect has filled by new formed bone and the quantity of newly formed bone in this group is more than control. Newly formed bone is more acidofilic because of active osteoid and its calcification. There is discontinuity and gap interface new and old

formed bone. In the medullary cavity of femoral bone, masses of bone cement which covered by connective tissue is obvious. H&E, 40x.

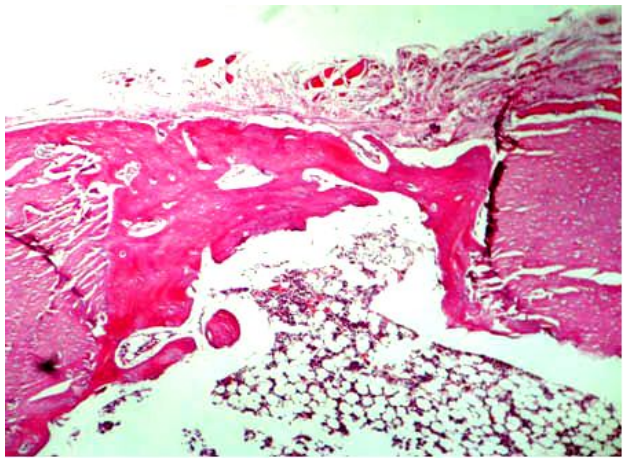

Fig 4: Microscopic view from healing area in the middle part of femoral diaphysis from group treated with Calcium Phosphate Granules. More advanced stage of remodeling and consolidation and development of haversian systems is obvious. Wide spaces of bone marrow are seen in the some areas of newly formed bone yet. H&E, 40x.

Histomorphometry Results

Histomorphometry evaluation results obtained, indicating that amounts of lamellar bone formed in experimental group significantly more control group (p=0/000) and is less than healthy bone (p=0/000). Amount of immature bone, bone marrow in experimental group significantly lower than control group, respectively (p=0/000). Mean and error bar than lamellar bone, immature bone, bone marrow among the studied groups, is presented in Fig. 5.

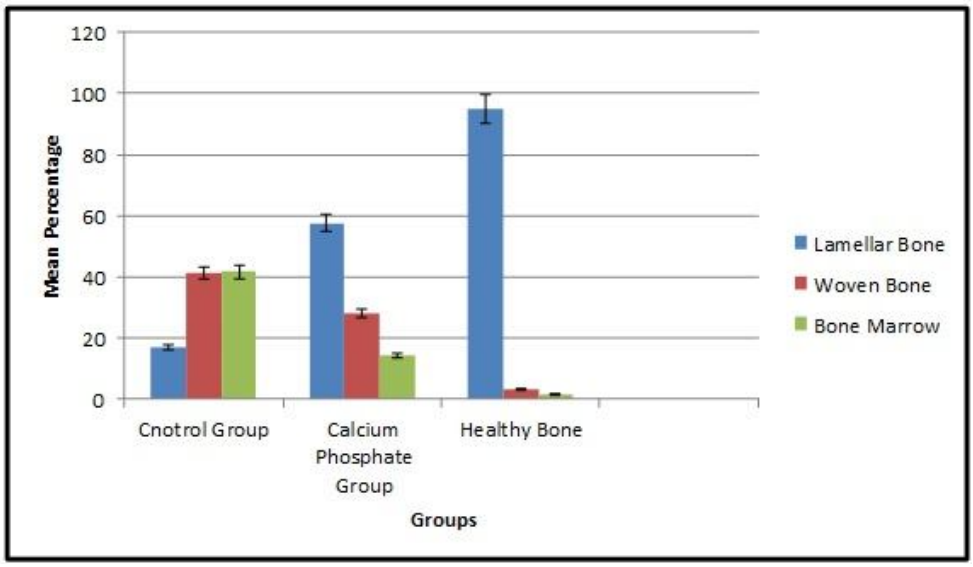

Fig 5: Chart component repair bone tissue status between the study groups- In bone samples obtained 45 days after surgery

DISCUSSION

An ideal material for bone healing should have osteoinductive and osteogenic properties (2). As a result, some investigators used mixtures of synthetic scaffolding biomaterials and osteoinductive organic agents to achieve better results (13). Calcium phosphate is new generation bone substitutes, with potential clinical applications in orthopaedics. Calcium phosphate granules have become materials of choice for bone repair, because of their biocompatibility and osteoconductivity (14). The current study aimed to evaluate the positive effect of calcium phosphate granules on bone healing of the femurs defect in rabbit.

Histopathological findings showed that bone defect in control group which was as an empty cavity and changing of connective tissue into the bone and its healing has not more severity and extension because of absence materials having growth promotion effects. Based on following healing pattern in the control group, can be stated that firstly, a lot of fibrinous clots and WBS fill the defect area then peripheral vessels and fibrous tissue penetrate into it. Formation of new bone and vessels starts from surround then progresses centrally to fill the defect. At the end of the study, control group had a lot of connective tissue in the defect area than treatment group, while, treatment group had bone union instead of undifferentiated mesenchymal tissue. Based on stimulatory nature of materials used and accelerating of histological process, bone trabeculae was formed more regular and organized than control group and defect was filled by newly formed bone and has prevented from formation of fibrous tissue in the defect area.

Histomorphometric evaluations showed that most of the defect area in the control group has been filled by immature bone and connective tissue and few amounts of lamellar bone, indicates more active osteogenesis in the treatment group. In the histopathologic evaluations of treatment group, there was bone union instead of mesenchymal tissue which has filled defect area. A lot of immature woven bone and a few mature bone trabeculae have been filled defect area which is changing into the lamellar bone. It seems that in the treatment group, there is a bed for osteogenic cells to facilitate them movement and progressing centrally development. Histopathologic and Histomorphometric evaluations in present study showed that calcium phosphate granules improve osteogenesis as seen in the treatment group. In a radiological study by Menon and Varma, 2005, they used of hydroxyapatite granules in healing of bone fractures in the 28 humans and it revealed that hydroxyapatite granules are bio-substances ease to use and have stimulatory effect on osteogenesis in the defect area (15).

Komaki and et al. showed that segmental bone defects were healed with cortical bone 12 weeks after implantation of the complex of β-TCP granules and 5% collagen with rhFGF-2. They showed that the resorption of β-TCP is important for bone formation (7). Fernandez showed that calcium phosphate is replaced by new bone, as the healing process progresses, this is the osteoconductive property which provides better strength to the healing site (16).

In a study by Ignjatovic et al., 2007, they used of Poly-D-, L- Lactide-co-Glycolide as bone replacement and they found that calcium phosphate and hydroxyapatite have most important effect on aggregation of fibroblasts and their union, which provides situation for healing in the defect area (17).

CONCLUSION

The results of this study indicate that calcium phosphate granules is a good choice for the healing of bone defects, and provides a more rapid regeneration of bone defects.

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